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Synthesis and biological evaluation of novel indolyl-dihydropyridin-3-carboxylate, dihydro[1,2,4]triazol[1,5]pyridin-3-carboxylate and carbohydrazone derivatives

Prabhaker Walmik

Department of Post-Graduate Studies and Research in Chemistry,
Sahyadri Science College (Kuvempu University), Shivamogga 577 203, India
E-mail: prabhakarchavan7@gmail.com

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The present study anticipates the development of novel antioxidant, antimicrobial and antitubercular drugs by using the indole scaffold. Novel indole derivatives, (**4a-c**), (**5a-c**) and (**6a-i**) have been prepared. The structures of all these unknown compounds have been confirmed with the help of elemental analysis, IR, ^1H & ^{13}C NMR and mass spectral data. All these previously unknown synthesized compounds screened for their antioxidant, antimicrobial and antitubercular activities. Compound **5a** exhibited good RSA 76.58 %, IC_{50} value <25 $\mu\text{g/mL}$. Compound **5a** exhibited higher absorbance 0.691nm, at concentration 100 $\mu\text{g/mL}$. Compound **6a** showed good metal chelating activity (64.10 %) at a concentration 100 $\mu\text{g/mL}$. Compound **6a** showed potent activity against *S. aureus* and *P. aeruginosa*. Compounds **4a** and **5a** showed promising antitubercular activity MIC 6.25 $\mu\text{g/mL}$. Compound **6a** showed excellent activity (MIC= 3.125 $\mu\text{g/mL}$) against *M. tuberculosis* H37Rv, than the standards pyrazinamide (MIC= 3.125) and Streptomycin (6.25 $\mu\text{g/mL}$).

Keywords: Indole, dihydropyridine, dihydro[1,2,4]triazol[1,5]pyridine, antimicrobial, antioxidant, antitubercular activity

Antioxidants are playing a vital role because of their involvement in biological and industrial processes. In general, compounds with antioxidant activity have been found to possess anti-cardiovascular, anti-inflammatory and anticancer¹⁻³ activities. Reactive oxygen species (ROS) and antituberculosis⁴. Free radicals are considered to be implicated in a variety of pathological events, such as cancer and aging agents⁵⁻⁷.

Indole derivatives constitute an important class of therapeutic agents in medicinal chemistry such as antimicrobial⁸, antioxidant⁹, antiviral¹⁰, anti-HIV, antimalarial¹¹ and dihydropyrimidine derivatives are widely used for the variety of pharmacological activities^{12,13}, antitubercular¹⁴, antitumor¹⁵, antiepileptic¹⁶, antimalarial¹⁷, antiviral¹⁸, anti-inflammatory¹⁹, analgesic²⁰ and antimicrobial²¹ activity.

The treatment of infectious diseases still remains an important and challenging problem because of a combination of factors including emerging infectious diseases and the increasing number of multi-drug resistant microbial pathogens. In spite of a large number of antibiotics and chemotherapeutics available for medicinal use, at the same time the emergence of resistance developed against old and new antibiotics in the last decades revealed a substantial need for new

classes of antimicrobial agents. There is really perceived need for the discovery of new compounds endowed with antimicrobial activity, possibly acting through mechanisms of action, which are distinct from those of well-known classes of antibacterial agents to which many clinically relevant pathogens are now resistant²².

There are many marketed drugs containing the 1,2,4-triazole group e.g., triazolam, alprazolam, etizolam and furacyclin. From the literature²², it may be predicted that 1,2,4-triazole moiety represents important pharmacophore and play a vital role in medicinal agents. A degree of respectability has been bestowed upon 1,2,4-triazole derivatives due to their wide range of biological activities such as antibacterial²³, antifungal²⁴, antitubercular²⁵, anticancer²⁶ anti-tumor²⁷ activity.

In view of the above findings and in continuation of our research on the synthesis of biologically active molecules²⁸⁻³² in present investigation we report the synthesis, antioxidant, antimicrobial and antitubercular activities of novel indole derivatives. In the design of new drugs, the combination of different pharmacophores frame may lead to compounds with interesting biological profile.

Results and Discussion

Chemistry

A mixture of 2-cyanoacetohydrazide (**1**), 2, 5-disubstituted indol-3-carboxaldehyde (**2**), ethyl-2-cyanoacetate (**3**) in ethanol using catalytic amount of acetic acid under reflux temperature to afforded ethyl 1, 2-diamino-4-(5-substituted-2-phenyl-1*H*-indo-3-yl)-5-cyano-6-oxo-1,6-dihydropyridin-3-carboxylates (**4a-c**). Compounds **4a-c** react with triethyl-orthoformate under reflux condition to gave Ethyl 7-(5-substituted-2-phenyl-1*H*-indo-3-yl)-5-cyano-6-oxo-1,5-dihydro[1,2,4]triazol[1,5]pyridin-3-carboxylates (**5a-c**). Compounds **5a-c** hydrazinolysis in boiling ethanol afforded the Ethyl 7-(5-chloro-2-phenyl-1*H*-indo-3-yl)-5-cyano-6-oxo-1,5-dihydro[1,2,4]triazol[1,5]pyridin-8-carbohydrazides (**6a-c**). Structure of all the newly synthesized compounds have been accomplished on the basis of elemental analyses and spectral techniques like IR, ¹H NMR, ¹³C NMR and Mass spectroscopy. The detailed synthetic strategy is outlined in (Scheme I). Analytical and spectral data of the synthesized compounds are given in the experimental section.

Biological activities

Antioxidant activities

1,1-Diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity (RSA)

Numbers of methods are available for the determination of free radical scavenging activity (RSA) but the assay employing the stable DPPH[•] has received much attention owing to its ease of use and convenience. This assay is the most widely used *in vitro* test to assess free radical scavenging capacities of test compounds. The RSA of synthesized

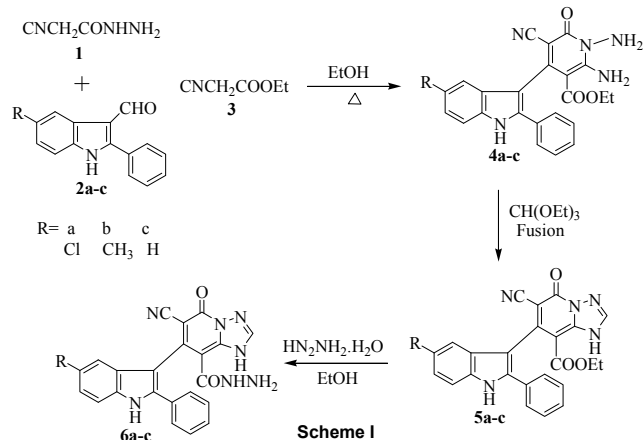
compounds was carried out at 25, 50, 75 and 100 µg/mL concentrations in methanol using DPPH method. All the analyses results performed on three replicate and the results were averaged. Results are expressed as percentage decrease with respect to the control values.

The results are illustrated in the Table I. Compounds **4a**, **5a**, **6a**, **4c**, **5c** & **6c**, showed good RSA (76.58, 73.82, 74.65, 70.24, 75.20 & 71.90%) at concentration 100 µg/mL. The best result was obtained by compound **5a** (76.58 %, IsC₅₀ value <25 µg/ml) when compared to standards BHA (92.28 %), TBHQ (91.46 %) and AA (94.21 %). This higher RSA may be attributed to the presence of two amino group and electronegative chlorine group present in it, which may be responsible for stabilization of free radical formed after donating a hydrogen atom to DPPH free radical. However, none of the compounds exhibited better activity than the standards.

Ferric ions (Fe³⁺) reducing antioxidant power (FRAP)

The reductive capacities of synthesized compounds were assessed by the extent of conversion of Fe³⁺/ferricyanide complex to the Fe²⁺/ferrous form. The reductive power of the compounds was observed at different concentrations and results were compared with standards BHA, TBHQ and AA. The reducing ability result was given in the Table II.

Compounds **4a**, **5a**, **5b**, **5c** and **6a** reduced metal ion complexes to their lower oxidation state or take part in electron transfer reaction. In other words, these compounds showed the ability of electron donor to scavenge free radicals. The rest of the compounds showed lower absorbance as compared to the



Scheme I — Synthetic approach for the preparation of the compounds (4-6a-c)

Table I — DPPH radical scavenging activity of compounds (4-6)

	Concentrations			
	25 µg/ml (%)	50 µg/ml (%)	75 µg/ml (%)	100 µg/ml (%)
4a	60.05	62.80	64.73	76.58
4b	52.34	55.64	60.05	64.18
4c	58.12	61.14	66.66	70.24
5a	64.46	65.84	68.59	73.82
5b	60.33	63.63	68.87	72.17
5c	61.98	64.73	68.31	75.20
6a	56.47	61.98	68.04	74.65
6b	54.26	60.33	62.25	68.04
6c	57.85	60.05	63.90	71.90
BHA	85.95	88.98	90.90	92.28
TBHQ	86.50	87.87	89.25	91.46
AA	88.15	90.08	92.56	94.21

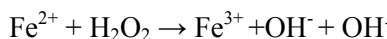
Table II — Ferric ions (Fe^{+3}) activity of compounds 4-6

Comp. No.	Concentrations			
	25 $\mu\text{g/ml}$ (nm)	50 $\mu\text{g/ml}$ (nm)	75 $\mu\text{g/ml}$ (nm)	100 $\mu\text{g/ml}$ (nm)
4a	0.255	0.393	0.519	0.621
4b	0.219	0.285	0.348	0.465
4c	0.268	0.299	0.345	0.485
4a	0.210	0.391	0.573	0.691
4b	0.221	0.278	0.394	0.534
4c	0.296	0.288	0.469	0.497
5a	0.277	0.398	0.478	0.686
5b	0.263	0.379	0.513	0.516
5c	0.190	0.362	0.495	0.589
BHA	0.860	0.910	1.101	1.289
TBHQ	0.802	0.949	1.101	1.295
AA	0.691	0.851	0.999	1.149

standards. The best result was obtained by compound **5a** higher absorbance 0.691nm, at concentration 100 $\mu\text{g/ml}$ when compared to standards BHA, TBHQ and AA. The higher the absorbance of the compounds indicated greater reducing power.

Ferrous (Fe^{2+}) metal ion chelating activity

Among the transition metals, iron is known as the most important lipid oxidation pro-oxidant due to its high reactivity. The effective ferrous ions chelators may also afford protection against oxidative damage by removing iron (Fe^{2+}) that may otherwise participate in hydroxyl radical generating Fenton type reactions³³.



Ferric (Fe^{3+}) ions also produce radical from peroxides although the rate is tenfold less than that of ferrous (Fe^{2+}) ion³⁴. Ferrous ion is the pro-oxidant among the various species of metal ions³⁵. Minimizing ferrous (Fe^{2+}) ion may afford protection against oxidative damage by inhibiting production of reactive oxygen species (ROS) and lipid production. Ferrozine can quantitatively form complexes with ferrous ions in this method. In the presence of chelating agents the complex formation is disrupted resulting in a decrease in red color of the complex. Measurement of color reduction therefore allows estimating the metal chelating activity of the co-existing chelators. Lower absorbance indicates higher metal chelating activity. The chelating effects of ferrous ions (Fe^{2+}) with test compounds were determined using standards BHA, TBHQ and AA.

In this assay, synthesized compounds interfered with the formation of ferrous and ferrozine complex. From the Table III it was concluded that, compounds

Table III — Metal chelating activity of compounds (4-6)

Comp. No.	Concentrations				IC_{50} ($\mu\text{g/ml}$)
	25 $\mu\text{g/ml}$ (%)	50 $\mu\text{g/ml}$ (%)	75 $\mu\text{g/ml}$ (%)	100 $\mu\text{g/ml}$ (%)	
4a	39.00	49.85	63.15	68.42	< 25
4b	28.17	34.05	41.17	48.91	< 25
4c	37.77	52.01	57.27	66.27	< 25
5a	42.41	49.53	56.65	64.70	< 25
5b	47.05	52.63	56.65	61.91	< 25
5c	39.00	49.22	52.94	58.82	< 25
6a	54.55	58.27	69.53	74.10	< 25
6b	41.79	48.29	50.77	61.91	< 25
6c	44.58	52.01	57.58	62.30	< 25
BHA	63.46	68.11	69.65	72.13	< 25
TBHQ	61.60	64.39	69.34	70.58	< 25
AA	63.15	65.63	70.58	72.44	< 25

4a, 5a, 6a, 4c, 5c and **6c** exhibited (68.42, 64.70, 74.10, 66.27, 61.91 and 62.30 %, IC_{50} value <25 $\mu\text{g/ml}$), respectively, these compounds exhibited good chelating activity and are able to capture ferrous ions before ferrozine.

Antimicrobial activity

Antimicrobial activity results (Table IV) the MIC value it is clear that most the tested compounds were active in the concentration range of 62.5–250 $\mu\text{g/mL}$ which is comparatively more or equipotent than the standards gentamycin and fluconazole. Antibacterial activity of screened samples, compound **4a** showed potent activity (62.5 $\mu\text{g/mL}$) against *Escherichia coli* (MTCC-723), **5a** showed potent activity (62.5 $\mu\text{g/mL}$) against *Pseudomonas aeruginosa*

(MTCC-1688) and **6a** showed potent activity (62.5 $\mu\text{g/mL}$) against *Staphylococcus aureus* (ATCC-29513) and *Pseudomonas aeruginosa* (MTCC-1688), this potent activity may be due to presence of chlorine atom at C-5 position of indole system. Remaining all the tested compounds exhibited equipotent or less potent activity than the standard. Compounds **4a, 5a** and **6a** exhibited equipotent activity against all the above four microorganisms when compare with the standard. Whereas, Rest of the compounds in the series exhibited moderate to less activity.

Antifungal activity screening results revealed that the compounds **4a** and **5a** showed potent activity (62.5 $\mu\text{g/mL}$) against *Aspergillus niger* (MTCC-281), **6a** showed potent activity (62.5 $\mu\text{g/mL}$) against *Aspergillus oryzae* (MTCC-3567^T), *Aspergillus flavus* (MTCC-1973) and *Aspergillus terreus* (MTCC-1782), this potent activity may be due to presence of

Table IV — *In-vitro* antimicrobial activities of compounds (4-6)

Com. code	Antibacterial activity (MIC µg/mL)				Antifungal activity (MIC µg/mL)			
	EC ^a	SA ^b	KP ^c	PA ^d	AO ^e	AN ^f	AF ^g	AT ^h
4a	62.5	125	125	125	125	62.5	125	250
4b	250	250	250	250	500	500	250	500
4c	125	500	500	500	500	250	125	500
5a	125	125	250	62.5	125	62.5	250	250
5b	500	250	500	250	250	125	250	500
5c	125	500	250	250	250	500	250	500
6a	125	62.5	250	62.5	125	125	125	125
6b	500	250	500	250	500	250	500	500
6c	250	500	500	250	250	125	250	500
Gentamycin	125	125	250	125	--	--	--	--
Fluconazole	--	--	--	--	125	62.5	125	250

chlorine atom at C-5 position of indole system. Whereas, Rest of the compounds in the series exhibited moderate to less activity.

Screening studies have demonstrated that the newly synthesized compounds have promising antibacterial and antifungal properties. Therefore, it was concluded that there exists better scope for further study on this class of compounds.

^aEC- *Escherichia coli* (MTCC-723), ^bSA- *Staphylococcus aureus* (ATCC-29513), ^cKP- *Klebsiella pneumonia* (NCTC-13368), ^dPA- *Pseudomonas aeruginosa* (MTCC-1688) ^eAO- *Aspergillus oryzae* (MTCC-3567^T), ^fAN- *Aspergillus niger* (MTCC-281), ^gAF- *Aspergillus flavus* (MTCC-1973), ^hAT- *Aspergillus terreus* (MTCC-1782).

Antitubercular activity

The results of the antitubercular evaluation results are given in (Table V). Newly synthesized compounds (**4-6**) were assayed for inhibitory activity towards *Mycobacterium tuberculosis* H37Rv (ATCC2794). The minimum inhibitory concentration (MIC expressed as µg/mL) was determined for each compound. The compound 6a showed excellent activity against *M. tuberculosis* H37Rv (MIC= 3.125 µg/mL) than the standards Pyrazinamide and Streptomycin (MIC= 3.125 and 6.25 µg/mL). Compounds **4a** and **5a** showed good activity against *M. tuberculosis* H37Rv (MIC= 6.25 µg/mL). Compounds **4b** and **5b** showed moderate activity against *M. tuberculosis* H37Rv (MIC= 12.5 µg/mL). In general, the brief structure-activity relationship (SAR) studies revealed that the presence of electron withdrawing group chlorine at C-5 indole system may be attributed for enhanced antitubercular

Table V — Antitubercular activity of compounds (4-6)

Comp. No.	MIC ^a values (µg/mL)
4a	3.125
4b	12.5
4c	25
5a	6.25
5b	12.5
5c	25
6a	6.25
6b	25
6c	25
Pyrazinamide	3.125
Streptomycin	6.25

activity in the series and has emerged as promising antitubercular agents.

Experimental Section

Chemistry

Materials and methods

All the reagents were obtained commercially and used by further purification using standard procedures. Melting points were determined by an open capillary method and are uncorrected. Purity of the compounds was checked by thin layer chromatography using silica gel-G coated Al plates (Merck) and spots were visualized by exposing the dry plates in iodine vapors. The IR (KBr pellet) spectra were recorded on a Perkin-Elmer (Spectrum ONE) FT-IR Spectrometer. The ¹H and ¹³C NMR (DMSO-*d*₆) spectra were recorded with a BRUKER NMR 500 and 125 MHz spectrometers, and the chemical shift values are expressed in ppm (δ scale) using tetramethylsilane as an internal standard. The mass spectral measurements were carried out by Electron Impact method on JEOL GC mate spectrometer at 70 eV. Elemental analyses were performed on flash EA 1112 series elemental analyzer.

5-Substituted 2-phenyl-1H-indol-3-carboxaldehydes (**2a-c**) were prepared by literature method [36].

General procedure for the synthesis of ethyl 1, 2-diamino-4-(5-substituted-2-phenyl-1H-indo-3-yl)-5-cyano-6-oxo-1,6-dihydropyridin-3-carboxylates (4a-c**):** mixture of compounds **1** (0.01 mol), 2,5-disubstituted indol-3-carboxaldehyde (0.01 mol) and ethyl-2-cyanoacetate (**3**) (0.01 mol) in ethanol (25mL) was refluxed for 5 h. The reaction mixture was cooled at room temperature and the solid obtained was filtered off, dried and crystallized to give compounds **4a-c**, respectively (**Scheme I**).

Ethyl 1, 2-diamino-4-(5-chloro-2-phenyl-1H-indo-3-yl)-5-cyano-6-oxo-1,6-dihydropyridin-3-

carboxylate (4a): Yield: 72%; m.p. 220-221°C; R_f 0.75 ethyl acetate: benzene (1:3) mixture; FT-IR (KBr) (cm^{-1}): 3380 (NH_2), 3289 (NH_2), 3188 (NH), 2198 ($\text{C}\equiv\text{N}$), 1771, 1684 (2 $\text{C}=\text{O}$), 1613 ($\text{C}=\text{C}$), 1568 ($\text{C}=\text{N}$), 674 ($\text{C}-\text{Cl}$); ^1H NMR ($\text{DMSO}-d_6$): δ 2.49 (t, $J = 8.83\text{Hz}$, 3H, CH_3), 3.68 (q, $J = 31.01\text{ Hz}$, 2H, CH_2CO), 5.77 and 8.77 (2s, 2H, 2NH), 7.62–8.38 (m, 8H, Ar-H), 11.10 (s, 1H, indole-NH); ^{13}C NMR ($\text{DMSO}-d_6$): δ 170.9, 166.6, 134.0, 132.8, 130.1, 129.5, 128.3, 128.1, 127.8, 127.5, 126.8, 123.9, 122.7, 121.5, 115.9, 114.5, 111.0, 89.5, 62.5 & 15.1; MS (EI) m/z 447 (M^+), 449 ($\text{M}^+ + 2$); Anal. Calcd. for $\text{C}_{23}\text{H}_{18}\text{N}_5\text{O}_3\text{Cl}$ (447.11): C, 61.68; H, 4.05; N, 15.64. Found: C, 61.71; H, 4.08; N, 15.71%.

Ethyl 1, 2-diamino-4-(5-methyl-2-phenyl-1H-indol)-5-cyano-6-oxo-1,6-dihydropyridin-3-carboxylate (4b): Yield: 76%; m.p. 265-266°C; R_f 0.71 ethyl acetate: benzene (1:3) mixture; FT-IR (KBr) (cm^{-1}): 3388 (NH_2), 3295 (NH_2), 3187 (NH), 2197 ($\text{C}\equiv\text{N}$), 1770, 1685 (2 $\text{C}=\text{O}$), 1617 ($\text{C}=\text{C}$), 1570 ($\text{C}=\text{N}$); ^1H NMR ($\text{DMSO}-d_6$): δ 1.39 (s, 1H, CH_3), 2.50 (t, $J = 8.83\text{Hz}$, 3H, CH_3), 3.67 (q, $J = 31.01\text{ Hz}$, 2H, CH_2CO), 5.72 and 8.70 (2s, 2H, 2NH), 7.57–8.34 (m, 8H, Ar-H), 11.14 (s, 1H, indole-NH); MS (EI) m/z 427 (M^+). Anal. Calcd. for $\text{C}_{24}\text{H}_{21}\text{N}_5\text{O}_3$ (427.16): C, 67.44; H, 4.95; N, 16.38. Found: C, 67.41; H, 4.98; N, 16.51%.

Ethyl 1, 2-diamino-4-(2-phenyl-1H-indol)-5-cyano-6-oxo-1,6-dihydropyridin-3-carboxylate (4c): Yield: 76%; m.p. 24-241°C; R_f 0.82 ethyl acetate: benzene (1:4) mixture; FT-IR (KBr) (cm^{-1}): 3381 (NH_2), 3292 (NH_2), 3189 (NH), 2192 ($\text{C}\equiv\text{N}$), 1769, 1680 (2 $\text{C}=\text{O}$), 1613 ($\text{C}=\text{C}$), 1568 ($\text{C}=\text{N}$); ^1H NMR ($\text{DMSO}-d_6$): 2.49 (t, $J = 8.83\text{Hz}$, 3H, CH_3), 3.67 (q, $J = 31.01\text{ Hz}$, 2H, CH_2CO), 5.77 and 8.79 (2s, 2H, 2NH), 7.64–8.39 (m, 9H, Ar-H), 11.12 (s, 1H, indole-NH); MS (EI) m/z 413 (M^+). Anal. Calcd. for $\text{C}_{23}\text{H}_{19}\text{N}_5\text{O}_3$ (413.15): C, 66.82; H, 4.63; N, 16.94. Found: C, 66.88; H, 4.68; N, 16.91%.

General procedure for the synthesis of ethyl 7-(5-unstituted-2-phenyl-1H-indo-3-yl)-5-cyano-6-oxo-1,5-dihydro[1,2,4]triazol[1,5]pyridin-3-carboxylates (5a-c):

A mixture of compounds (4a-c) (0.01 mol) and triethyl orthoformate (0.02 mol) was refluxed for 3 h. The reaction mixture was cooled and the solid obtained was filtered off, dried and crystallized to give compounds 5a-c, respectively (Scheme I).

Ethyl 7-(5-chloro-2-phenyl-1H-indo-3-yl)-5-cyano-6-oxo-1,5-dihydro[1,2,4]triazol[1,5]pyridin-

3-carboxylate (5a): Yield: 65%; m.p. 187-189°C; R_f 0.78 ethyl acetate: toluene (1:4) mixture; FT-IR (KBr) (cm^{-1}): 3188 (NH), 3160 (NH), 2198 ($\text{C}\equiv\text{N}$), 1771, 1684 (2 $\text{C}=\text{O}$), 1613 ($\text{C}=\text{C}$), 1568 ($\text{C}=\text{N}$), 674 ($\text{C}-\text{Cl}$); ^1H NMR ($\text{DMSO}-d_6$): δ 2.50 (t, $J = 8.81\text{Hz}$, 3H, CH_3), 3.66 (q, $J = 31.03\text{ Hz}$, 2H, CH_2CO), 5.71 (s, 1H, NH), 7.64–8.39 (m, 8H, Ar-H), 11.12 (s, 1H, indole-NH); ^{13}C NMR ($\text{DMSO}-d_6$): δ 170.3, 166.2, 160.8, 154.0, 135.3, 131.7, 129.8, 129.5, 127.8, 127.4, 125.9, 123.6, 122.8, 121.5, 115.9, 114.4, 110.8, 90.1, 63.3 & 15.2; MS (EI) m/z 457 (M^+), 459 ($\text{M}^+ + 2$). Anal. Calcd. for $\text{C}_{24}\text{H}_{16}\text{N}_5\text{O}_3\text{Cl}$ (457.09): C, 62.96; H, 3.52; N, 15.30. Found: C, 62.98; H, 3.58; N, 15.38%.

Ethyl 7-(5-methyl-2-phenyl-1H-indo-3-yl)-5-cyano-6-oxo-1,5-dihydro[1,2,4]triazol[1,5] pyridin-3-carboxylate (5b): Yield: 67 %; m.p. 171-172°C; R_f 0.79 ethyl acetate: toluene (1:3) mixture; FT-IR (KBr) (cm^{-1}): 3190 (NH), 3168 (NH), 2191 ($\text{C}\equiv\text{N}$), 1767, 1680 (2 $\text{C}=\text{O}$), 1611 ($\text{C}=\text{C}$), 1565 ($\text{C}=\text{N}$); ^1H NMR ($\text{DMSO}-d_6$): δ 1.39 (s, 1H, CH_3), 2.54 (t, $J = 8.80\text{Hz}$, 3H, CH_3), 3.68 (q, $J = 31.04\text{ Hz}$, 2H, CH_2CO), 5.69 (s, 1H, NH), 7.60–8.39 (m, 8H, Ar-H), 11.14 (s, 1H, indole-NH); MS (EI) m/z 437 (M^+). Anal. Calcd. for $\text{C}_{25}\text{H}_{19}\text{N}_5\text{O}_3$ (437.15): C, 68.64; H, 4.38; N, 16.01. Found: C, 68.68; H, 4.35; N, 16.14%.

Ethyl 7-(2-phenyl-1H-indo-3-yl)-5-cyano-6-oxo-1,5-dihydro[1,2,4]triazol[1,5]pyridin-3-carboxylate (5c): Yield: 69 %; m.p. 144-145°C; R_f 0.75 ethyl acetate: toluene (1:1) mixture; FT-IR (KBr) (cm^{-1}): 3198 (NH), 3172 (NH), 2164 ($\text{C}\equiv\text{N}$), 1769, 1677 (2 $\text{C}=\text{O}$), 1613 ($\text{C}=\text{C}$), 1569 ($\text{C}=\text{N}$); ^1H NMR ($\text{DMSO}-d_6$): δ : 2.55 (t, $J = 8.80\text{Hz}$, 3H, CH_3), 3.67 (q, $J = 31.04\text{ Hz}$, 2H, CH_2CO), 5.71 (s, 1H, NH), 7.59–8.41 (m, 9H, Ar-H), 11.17 (s, 1H, indole-NH); MS (EI) m/z 423 (M^+). Anal. Calcd. for $\text{C}_{24}\text{H}_{17}\text{N}_5\text{O}_3$ (423.13): C, 68.08; H, 4.05; N, 16.54. Found: C, 68.10; H, 4.15; N, 16.60%.

General procedure for the synthesis of ethyl 7-(5-substituted-2-phenyl-1H-indo-3-yl)-5-cyano-6-oxo-1,5-dihydro[1,2,4]triazol[1,5] pyridin-8-carbohydrazides (6a-c):

A mixture of compounds (5a-c) (0.01 mol) and hydrazine hydrate 98% (0.015 mol) in tetrahydrofuran (20 mL) was heated under reflux condition for 5 h and then allowed to cool. The Precipitate that formed was filtered off, dried and crystallized to give compounds 6a-c, respectively (Scheme I).

Ethyl 7-(5-chloro-2-phenyl-1H-indo-3-yl)-5-cyano-6-oxo-1,5-dihydro[1,2,4]triazol[1,5] pyridin-8-carbohydrazide (6a): Yield: 56%; m.p. 184-185

°C; R_f 0.88 ethyl acetate: benzene (1:1) mixture; FT-IR (KBr) (cm^{-1}): 3380 (NH_2), 3302 (CONH), 3185 (NH), 3166 (NH), 2190 ($\text{C}\equiv\text{N}$), 1770, 1654 (2 $\text{C}=\text{O}$), 1611 ($\text{C}=\text{C}$), 1556 ($\text{C}=\text{N}$), 677 ($\text{C}-\text{Cl}$); ^1H NMR ($\text{DMSO}-d_6$): δ 5.12 (s, 1H, NH_2), 5.71 (s, 1H, NH), 7.44–8.40 (m, 8H, Ar-H), 9.81 (s, 1H, amide-NH), 11.17 (s, 1H, indole-NH); ^{13}C NMR ($\text{DMSO}-d_6$): δ 170.4, 166.3, 161.1, 153.0, 133.8, 132.1, 129.4, 128.9, 127.9, 127.8, 127.5, 125.9, 123.9, 122.6, 121.8, 115.9, 114.3, 111.1, 92.2, 63.5 & 15.3; MS (EI) m/z 443 (M^+), 445 (M^{+2}); Anal. Calcd. for $\text{C}_{22}\text{H}_{14}\text{N}_7\text{O}_2\text{Cl}$ (443.09): C, 59.53; H, 3.18; N, 22.09. Found: C, 59.64; H, 3.30; N, 22.14%.

Ethyl 7-(5-methyl-2-phenyl-1H-indo-3-yl)-5-cyano-6-oxo-1,5-dihydro[1,2,4]triazol[1,5] pyridin-8-carbohydrazide (6b): Yield: 52%; m.p. 154–155°C; R_f 0.71 ethyl acetate: benzene (1:2) mixture; FT-IR (KBr) (cm^{-1}): 3384 (NH_2), 3300 (CONH), 3188 (NH), 3167 (NH), 2195 ($\text{C}\equiv\text{N}$), 1773, 1658 (2 $\text{C}=\text{O}$), 1618 ($\text{C}=\text{C}$), 1554 ($\text{C}=\text{N}$); ^1H NMR ($\text{DMSO}-d_6$): δ 1.41 (s, 1H, CH_3), 5.14 (s, 1H, NH_2), 5.68 (s, 1H, NH), 7.43–8.39 (m, 8H, Ar-H), 9.81 (s, 1H, amide-NH), 11.09 (s, 1H, indole-NH); MS (EI) m/z 423 (M^+). Anal. Calcd. for $\text{C}_{23}\text{H}_{17}\text{N}_7\text{O}_2$ (423.14): C, 65.24; H, 4.05; N, 23.16. Found: C, 65.26; H, 4.10; N, 23.24%.

Ethyl 7-(2-phenyl-1H-indo-3-yl)-5-cyano-6-oxo-1,5-dihydro[1,2,4]triazol[1,5]pyridin-8-carbohydrazide (6c): Yield: 56%; m.p. 178–179°C; R_f 0.74 ethyl acetate: benzene (1:3) mixture; FT-IR (KBr) (cm^{-1}): 3389 (NH_2), 3306 (CONH), 3182 (NH), 3165 (NH), 2191 ($\text{C}\equiv\text{N}$), 1769, 1652 (2 $\text{C}=\text{O}$), 1612 ($\text{C}=\text{C}$), 1550 ($\text{C}=\text{N}$); ^1H NMR ($\text{DMSO}-d_6$): δ 5.61 (s, 1H, NH), 7.40–8.44 (m, 9H, Ar-H), 9.89 (s, 1H, amide-NH), 11.10 (s, 1H, indole-NH); MS (EI) m/z 409 (M^+). Anal. Calcd. for $\text{C}_{22}\text{H}_{15}\text{N}_7\text{O}_2$ (443.09): C, 64.54; H, 3.69; N, 23.95. Found: C, 64.64; H, 3.70; N, 23.99%.

Antioxidant activity assay

1, 1-Diphenyl-2-Picryl Hydrazyl (DPPH) Radical Scavenging Activity (RSA)

The free radical scavenging activity (RSA) of compounds (**4-6**) at concentration (25, 50, 75 and 100 $\mu\text{g/mL}$) was carried out in the presence of freshly prepared solution of stable free radical DPPH (0.04% w/v) following Hatano's method³⁷ using 2-tert-butyl-4-methoxyphenol (butylatedhydroxy anisole, BHA), 2-(1,1-dimethylethyl)-1,4-benzenediol (2-tert-butyl hydroquinone, TBHQ) and Ascorbic acid (AA) as standards. All the test analyses were performed on

three replicates and results are averaged. The results in percentage are expressed as the ratio of absorption decrease of DPPH in the presence test compounds and absorption of DPPH in the absence of test compounds at λ 517 nm on ELICO SL 171 Mini Spec spectrophotometer. The percentage scavenging activity of the DPPH free radical was measured using the following equation:

$$\% \text{ of DPPH RSA} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

The results are shown in the (Table I).

Ferric ions (Fe^{3+}) reducing antioxidant power (FRAP)

The Ferric ions (Fe^{3+}) reducing antioxidant power (FRAP) of the synthesized compounds (**4-6**) was determined according to the literature method³⁸. Different concentration of samples (25, 50, 75 and 100 $\mu\text{g/mL}$) in DMSO (1 mL) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH=6.6) and potassium ferricyanide (2.5 mL 1%). The mixture was incubated at 50°C for 20 min. After which a portion of trichloroacetic acid (2.5 mL, 10%) was added to the mixture and centrifuged for 10 min, at 1000 Xg. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.1%). Then absorbance at λ 700 nm was measured in spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power. The results are shown in the (Table II).

Ferrous (Fe^{2+}) metal ion chelating activity

The chelating activity of ferrous ion by synthesized compounds (**4-6**) was estimated by following reported method³⁹. The test samples (25, 50, 75 and 100 $\mu\text{g/mL}$) in ethanolic solution (0.4 mL) were added to a solution of FeCl_2 (0.05 mL, 2 mM). The reaction was initiated by the addition of ferrozine (0.2 mL, 5 mM) and the total volume was adjusted to 4 mL with ethanol. Ferrozine reacted with the divalent iron form stable magenta complex species that were very soluble in water. The mixture was shaken vigorously and kept at room temperature for 10 min. Then the absorbance of the solution was measured spectrophotometrically at λ 562 nm. All test analyses were run in triplicate and averaged. The percentage of inhibition of the ferrozine Fe^{2+} complex formations was calculated using the following formula:

$$\% \text{ of Ferrous ion Chelating} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

The control contains FeCl_2 and ferrozine, complex formation molecule. The results are shown in the (Table III).

In-vitro antimicrobial activity

The in vitro antimicrobial activity of all the synthesized compounds (4-6) was carried out by broth micro dilution method⁴⁰ in DMF at concentration 500, 250, 125 and 62.5 $\mu\text{g/mL}$. Muller hinton broth was used as nutrient medium to growth and dilutes the compound suspension for the test bacteria and Saboured Dextrose broth used for fungal nutrition. Inoculums size for test strain was adjusted to 10^8 CFU [Colony Forming Unit] per milliliter by comparing the turbidity. The strain employed for the activity was procured from Department of Biotechnolgy, Sahyadri Science College, Shivamoga.

The compounds (4-6) were screened for their antibacterial activity against *Escherichia coli* (MTCC-723), *Staphylococcus aureus* (ATCC-29513), *Klebsiella pneumonia* (NCTC-13368) and *Pseudomonas aeruginosa* (MTCC-1688), as well antifungal activity against *Aspergillus oryzae* (MTCC-3567^T), *Aspergillus niger* (MTCC-281), *Aspergillus flavus* (MTCC-1973) and *Aspergillus terreus* (MTCC-1782). DMSO used as a vehicle to get desired concentration of compounds to test upon microbial strains. The lowest concentration which showed no visible growth after spot subculture was considered as MIC for each compound. The standard antibiotics used for comparison in present study were gentamycin for evaluating for antibacterial activity as well as and fluconazole for antifungal activity. The protocols are summarized in (Table IV).

Antitubercular activity using Alamar Blue Dye

The antitubercular activity of compounds (4-6) was assessed against *M. tuberculosis* H37R_v strain using micro plate alamar blue dye assay (MABA)⁴¹. Briefly, 200 μL of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimize evaporation of medium in the test wells during incubation. The 96 wells plate received 100 μL of the middle brook 7H9 broth and serial dilution of compounds was made directly on plate. The final drug concentrations tested were 100 to 0.2 $\mu\text{g/mL}$ and compared with standards pyrazinamide 3.125 $\mu\text{g/mL}$ and streptomycin 6.25 $\mu\text{g/mL}$. Plates were covered and sealed with parafilm and incubated at 37°C for five days. After this time, 25 μL freshly prepared 1:1 mixture of almar blue reagent and 10 % tween-80 was

added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC (Minimal inhibition concentration) was defined as lowest drug concentration which prevented the color change from blue to pink. The results are shown in the (Table V).

Conclusions

In summary, few novel indole derivatives were prepared, in moderate to high yields. Our antioxidant, antimicrobial and antitubercular activities results proved that the chlorine substituent is essential to exhibit better activity. The presence of chlorine electron-withdrawing group on the indole system mostly favors the activity. Screening studies have demonstrated that the newly synthesized compounds have promising antimicrobial and antitubercular properties. Therefore, it was concluded that there exists better scope for further study on this class of compounds. Based on these excellent results some of the compounds will be screened for anticancer activity which will be reported in due course.

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